BIOCHEMICAL RESPONSE AND BIOMASS PRODUCTION OF *RHIZOPHORA MANGLE* L. TO EXPOSURE TO 2,4-DICHLOROPHENOXYACETIC ACID, UNDER EXPERIMENTAL CONDITIONS

CHAN-KEB, C. A.[1] – RUIZ-HERNANDEZ, J.[1] – DZUL-DECENA, M. N.[1] – PÉREZ-BALAN, R. A.[1] – GUTIÉRREZ-ALCÁNTARA, E. J.[2] – AGRAZ-HERNÁNDEZ, C. M.[2*]

[1]*Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Campeche, Avenida Ing. Humberto Lanz Cárdenas S/N, Colonia Ex Hacienda Kalá, C.P. San Francisco de Campeche 24085, Campeche, México*

[2]*Instituto EPOMEX, Universidad Autónoma de Campeche, Av. Héroe de Nacozari #480, Campus 6 de Investigaciones. C.P. San Francisco de Campeche 24029, Campeche, México*

**Corresponding author*
e-mail: clmagraz@uacam.mx

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Abstract. Anthropogenic activities have led to the degradation of mangrove ecosystems worldwide. The impact of herbicides reduces the survival of seedlings. Herbicides reduce the survival of seedlings by modifying the interstitial water's hydrological, soil, and physicochemical conditions, thus representing a latent risk in the environmental services these ecosystems provide. In this research, the behavior of biochemical parameters (total nitrogen, total protein, flavonoids), chlorophyll a, lipids, and the phytotoxic effect on the production of total biomass in *Rhizophora mangle* seedlings exposed to concentrations of 5 w/v dilutions (0%, 5%, 10%, 25%, 50% and 100%) of 2,4-Dichlorophenoxyacetic acid (2,4-D) in propagules grown in greenhouses was evaluated. Three phases were monitored: (1) stabilization of the water recirculation system, homogenization of interstitial water and adjustment of the artificial tide; (2) afforestation and conditioning; (3) application of 2,4-D acid, and monitoring of the concentration of biochemical parameters, chlorophyll a, lipids, total biomass and physicochemical parameters of interstitial water. The results showed that increasing the concentrations of 2,4-D generates a decrease in the concentrations of the biochemical variables, chlorophyll a, total biomass, and an increase in the concentration of fat in the seedlings, where variations were observed showing significant differences ($p < 0.05$). An inverse relationship was presented between the biochemical variables in chlorophyll a, total biomass production, and fat concentration. The research showed that *Rhizophora mangle* seedlings are very vulnerable to different concentrations of 2,4-D and to the developmental conditions to which they were exposed in this research.
Keywords: *flavonoids, seedlings, pollution, chlorophyll, phytotoxicity*

Introduction

Mangroves are productive ecosystems, they play a fundamental role in the protection of the coastline, and the prevention of floods, hurricane, and tsunami barriers; they are also part of the feeding, shelter, and growth of wildlife species (Troche-Souza et al., 2017), it is an ecosystem that prevents soil erosion through its aerial roots, it works as a filter that allows removing nutrients, toxins, accumulating carbon dioxide.

Mangroves are productive ecosystems, they play a fundamental role in the protection of the coastline, and the prevention of floods, hurricane, and tsunami barriers. They also provide feeding, shelter, and habitat for wildlife species (Troche-Souza et al., 2017). It is an ecosystem that prevents soil erosion through its aerial roots, it works as a filter that allows removing nutrients, toxins, and also sequestring carbon dioxide. The biodiversity

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of mangroves allows activities such as fish reproduction, mollusks, wood production, and fuels, among others. In Mexico, mangrove forests are located on the slopes of the Pacific, the Gulf of Mexico, and the Gulf of California (Velázquez-Salazar et al., 2021). Four types of species are known in Mexico: *Rhizophora mangle L, Avicennia germinans L*, *Laguncularia racemosa (L.) C.F. Gaertn* and *Conocarpus erectus L*.

The state of Campeche concentrates the largest extension of mangroves in the country (25.2%) (Troche-Souza et al., 2017). Mangroves have been transformed and are seriously threatened by urbanization and human activities, which receive various chemical, physical, and microbiological pollutants that alter their ecosystem, leading to low coastal protection against flooding. In the last 50 years, approximately one-third of the world's mangrove forests have been lost due to anthropogenic activities such as tourism, poorly developed or unsustainable aquaculture, immoderate industrial and urban development, development of tourist areas, high concentrations of nutrients, road construction, sewage discharges, etc. as well as unsustainable livestock and agricultural practices (Alongi, 2002; Zheng et al., 2000). The concentration of herbicides that are discharged in soils near mangrove forests is determined by factors such as the dose applied in the agricultural area, dilution in the soil due to the effect of rain and tide (Teas, 1976). Chemicals such as heavy metals, oil spills, and herbicides in tropical and subtropical habitats can reduce seedling survival (Peters et al., 1997; Giri et al., 2011; Lee, 1999). There is concern about the use of agrochemicals, and pesticides, their harmful effects, and the deterioration of the environment. However, the demand for pesticides has increased; and their extensive use is the main source of soil, water, and plant pollution. 2,4-D is one of the selective herbicides used at low concentrations to control broadleaf weeds. At high concentrations, it modifies normal growth and causes lethal damage to the plant (Pazmiño et al., 2011). However, even if applied to leaves, it can be absorbed through the roots and stems (Beyond Pesticides, 2025; Medina Méndez et al., 2018). It is the most widely used worldwide in Latin America and the United States in domestic environments, gardening, and as a herbicide in agricultural practices (Freisthler et al., 2022). According to the WHO (2009) various amounts of 2,4-Dichlorophenoxyacetic acid products applied to a given area can be distributed in the environment in a few hours or days by the movements of air, water or soil, particularly during periods of rain, strong winds or high temperatures. 2,4-D acid and its derivatives are broken down fairly quickly by hydrolysis, photolysis, and biological action. Arellano Aguilar and Rendón Von Osten (2016), analyzed the concentration of pesticides in the coastal zone, mentioning that this concentration may be due to the possible impact that pesticides transported by runoff with discharge to the sea could have. 2,4-D is toxic to aquatic plants and is more toxic to vascular plants than to nonvascular plants (Beyond Pesticides, 2025). On the other hand, Chan Keb et al. (2021) and previously Ivens (1957) reports the presence of 2,4-D in mangroves, the first signs observed were wilted leaves, and the defoliation of mangrove seedlings. Likewise, mangrove species (*Avicennia marina, Aegiceras corniculatum L*) are more tolerant to exposure to metals and herbicides, these species excrete salts in their stems and leaves, while species such as *Rhizophora stylosa, Ceriops australis* are considered mangrove plants that exclude sea salts from their tissues, are less tolerant to the aforementioned pollutants (Peng, 1997; MacFarlane, 2002; Bell and Duke, 2005). Despite the above, there is little research that evaluates the impact of pesticides on biomass production and the biochemical effects on mangrove species. Consequently, our objective was to determine the response (phytotoxic effect) to the exposure of 2,4-dichlorophenoxyacetic acid to different concentrations in the production of total biomass, chlorophyll *a,* total nitrogen, total protein, flavonoids, and lipids in *Rhizophora mangle* seedlings, under experimental conditions.

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Materials and methods

Description of the Rhizophora mangle hypocotyl collection area

The collection of biological material was carried out in the "Los Petenes" Biosphere Reserve (RBLP), located in the northwest of the Yucatan Peninsula, in the Gulf of Mexico, and the coastal area north of the State of Campeche (Rivera Arriaga et al., 2021), with an area of 282,857 ha (CONANP, 2006) (*Fig. 1*).

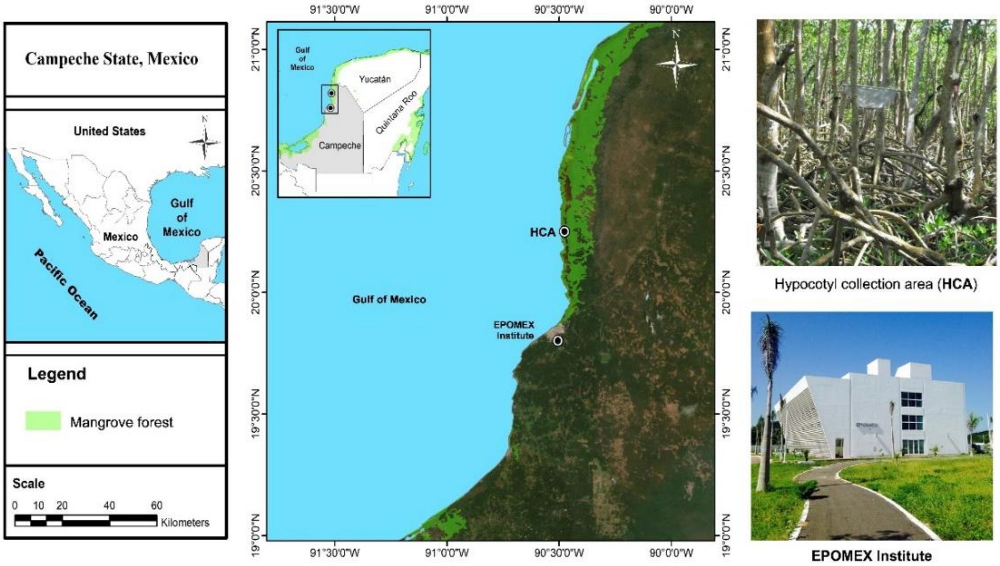

Figure 1. *Geographic location of the Rhizophora mangle hypocotyl collection forest in the Los Petenes Biosphere Reserve (RBLP), Campeche, Mexico, and experiment development sites (EPOMEX Institute). (Source: Chan-Keb et al., 2021)*

This area has a warm and sub-humid climate, with an average monthly temperature of 26°C and an average annual rainfall of 819 mm (Armitage et al., 2017). With Karst and hill-type soils, called Solonchak and to a lesser extent Gleysoles and Histosols, a karso-tectonic earth system (World Reference Base for Soil Resources (WRB) or the USDA Soil Taxonomy), the main morphological expression of plains, geomorphological landscapes, coastal marsh with large petenes, coastal marsh with white marshes and a degree of recent karst evolution (Bautista et al., 2005).

At the national and international level, the RBLP is considered of great relevance as it is part of an ecoregion that involves the Ría Celestún Biosphere Reserve and the El Palmar state protected natural area, in the state of Yucatán, as it is located in the Priority Regions of Mexico in all the categories of "Terrestrial Priority Regions No. 145, Priority Marine Regions No. 60, Priority Hydrological Regions No. 102, Areas of Importance for the Conservation of Birds-AICAS (AICAS SE-28) and for being declared in 2004, as RAMSAR site No. 1354 ha (CONANP, 2006). In the RBLP he registers four species of mangroves; *Avicennia germinans L., Laguncularia racemosa (L.) C.F. Gaertn, Conocarpus erectus L. and Rhizophora mangle L.* (Vázquez-Lule et al., 2019). *Rhizophora mangle* develops in habitats with constant freshwater input, tidal influence, and frequency, preferably in coastal environments with low wave energy (Naidoo, 2016). It is dominant along the coastline, forming monospecific forests; while, inland, it is common to be associated with other species (Chan-Keb et al., 2018). It is viviparous and

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produces propagules that germinate in the mother tree and its dispersal is through the tide, birds, and crustaceans. The propagule formation time is between 4 to 7 months (Gill and Tomlinson, 1997).

Despite the above, the northern area of the RBLP exhibits deterioration derived from human settlements in the urban area in the city of Campeche, due to urban and agricultural wastewater inputs (Velázquez-Salazar et al., 2021; Cissell et al., 2018). Likewise, more than 6000 ha of mangrove deaths are recorded in the central part of the Reserve, adjacent to the island of Jaina caused by the construction and expansion of communication routes, fires, and illegal logging (Velázquez-Salazar et al., 2021).

Construction and environmental stabilization of the system

In a greenhouse of 51.7 m^2 and 2 m high, the system was built with twelve Rotoplas® tanks (Grupo Rotoplas SAB de CV, Mexico City. Mexico) with a capacity of 450 L made of linear low-density polyethylene material. Hydraulic systems were installed for each tank to facilitate the simulation of the local tidal regime, high tide was simulated by gravity, and low tide using pumping (*Fig. 2*). The installation of the system was carried out taking care of air circulation and temperature fixation following the indications of Chan-Keb et al. (2021). The filters of each tank were constructed with 0.16 m^3 of mangrove peat, 0.13 m^3 of sand, and 0.13 m^3 of gravel. Mangrove peat was composed of 55% silt, 42% sand, and 3% clay, and was classified with a silty loam texture. Adjustments to the system such as topography, flood frequency, interstitial water salinity, and redox potential to simulate the plant collection site were made following historical records in Agraz-Hernández et al. (2018).

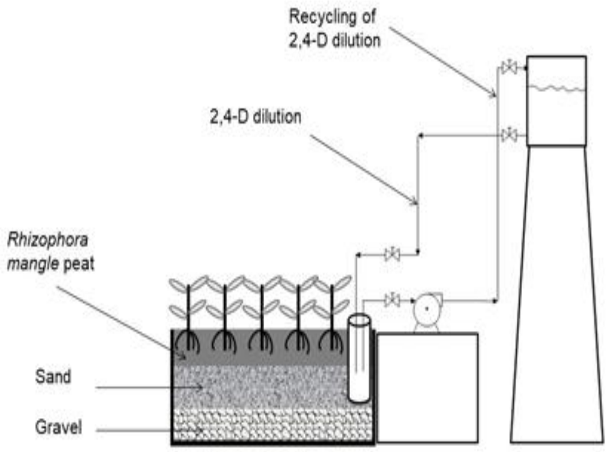

Figure 2. *Diagram of the treatment system for exposure of 2,4-D with subsurface recirculation. (Source: Chan-Keb et al., 2021)*

During 50 days, the physicochemical parameters of the water were monitored and afforestation was carried out, ensuring that the conditions were similar to the natural environment (*Fig. 3*).

Reforestation and conditioning stages

Bioassays and sample analysis were performed at the EPOMEX Institute of the Autonomous University of Campeche, Mexico. The collection of hypocotyls from

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Rhizophora mangle was carried out in September 2018, and the collection and analysis of the samples was carried out in April 2019 during the development of the Phase 3 experiment (*Fig. 3*).

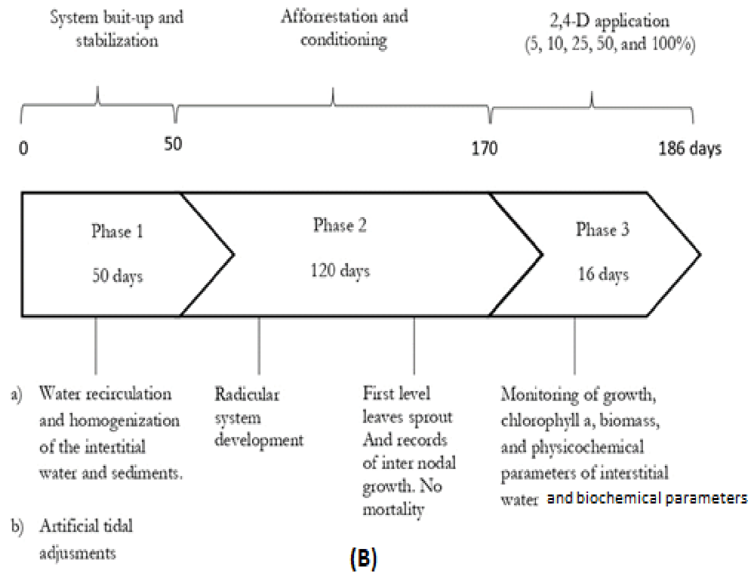

Figure 3. *Experiment timeline events. (Source: Chan-Keb et al., 2021)*

Mangrove hypocotyls were collected from a monospecific forest located in the RBLP (19°57′26.108” N, 90°26′59.40” W (*Fig. 1*) in the state of Campeche, in the Gulf of Mexico. Hypocotyls were selected and those that showed signs of plague, drying, or physical damage were excluded. The hypocotyls were seeded at a density of 65 per tank. The development of the root system and the first shoot leaf were observed over a period of 120 days. During this stage, physicochemical data were collected and tested using a one-way analysis of variance (one-way ANOVA) considering the data from the individual tanks and compared with each other, and no significant differences were found ($p > 0.05$). Internodal growth was recorded in all tanks and no plant mortality was detected at this stage. Later we established that the conditions were homogeneous, favoring nutrient absorption by the *R. mangle* seedlings and that they were ready for exposure to 2,4-D.

Application of 2,4-dichlorophenoxyacetic (2,4-D)

A commercial presentation of 2,4-D called “Full-Mina” (Dow AgroSciences de Colombia SA, Bogotá. Colombia) and considering the common practice of farmers to spread the pesticide, aliquots were diluted in tanks of 450 L of the contents of the pesticide bottle (480 g/l concentration of active ingredient); was assume a concentration of a dilution of 100% (10.8 g/L). The active ingredient of 2,4-D used in this experiment is the 2,4-D dimethylamine salt (DMA), CAS number: 2008-39-1, the agrochemical has an estimated half-life in an anaerobic aquatic laboratory between 41 and 333 days.

Five treatments and one negative control were performed in duplicate for a total of 12 experimental units (UE). Then, 5 different dilutions of 2,4-D were performed with seawater at weight/volume concentrations of 5%, 10%, 25%, 50%, and 100% (0.54, 1.08, 2.7, 5.4, and 10.8 g/L, respectively).

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Effects on the growth of Rhizophora mangle seedlings

The response of biomass production in seedlings was recorded at 12 days after exposure to 2,4-D. Three plants were extracted from each tank with their respective replicas to measure the total production of biomass. The stem, leaves, and roots of these plants were washed to remove the possible substrate adhered to the root, dried with brown paper, and dehydrated at 65°C in a convection oven for three days, once dehydrated, each of the components of the plant tissue were weighed (Agraz Hernández et al., 2011).

Chlorophyll a concentration

The determination of chlorophyll *a* was carried out using the technique proposed by Hansmann (Hansmann and America, 1973). The determination of chlorophyll concentration was carried out by collecting fresh leaves from three seedlings in each EU. First, 2 g of the fresh leaf was weighed and deposited in 15 mL test tubes, then crushed and 10 mL of 90% acetone (v/v) was added, stirred, and left to stand in darkness at 4°C for 24 h, then brought to room temperature and centrifuged at 2700 rpm for 5 min. Subsequently, the absorbances of the supernatant of each sample were measured using a 6500 spectrophotometer (JENWAY Stafford. United Kingdom) at wavelengths of 665 nm, 645 nm, and 630 nm. Ninety percent acetone was used as the target of the reagent. The concentration of chlorophyll *a* was determined based on the equation provided by Strickland and Parsons (1965). Results for chlorophyll concentration in mg/L were estimated per gram of tissue by fresh weight.

$$\text{Chlorophyll } a \text{ (mg/L)} = 11.6 \times (\text{Abs. } 665) - 1.31 \times (\text{Abs. } 645) - 0.14 \times (\text{Abs. } 630)$$

where Abs = is the absorbance of the respective wavelengths.

Flavonoid quantification

The quantification of flavonoids was quantified by the colorimetric method of aluminum chloride, the methodology of Pacheco et al. (2013) was followed. 1 g of dried leaf was taken and extracted with the soxhlet method with 100 mL ethanol for 1 h, and the extract was filtered and brought to a volume of 100 mL. Of the 100 mL of extract, 1 mL was taken and 5 mL of distilled water, 0.3 mL of $NaNO_2$ (1:20) were added; after 5 min 0.3 ml of $AlCl_3$ was added (1:10), and after 6 min 2 ml of NaOH was added and a volume of 10 mL was added with distilled water.

For quantification, a calibration curve of Quercetin (Sigma Aldrich) was developed at concentrations from 0 to 250 ppm. For the standard solution, 0.08 g of Quercetin was taken diluting in 100 mL of 96% ethanol, taking 1 mL and measuring 100 mL with 96% ethanol. Absorbances were measured using a 6500 spectrophotometer (JENWAY Stafford. United Kingdom) at wavelengths of 250 nm and 415 nm. The total flavonoid content was expressed as equivalent milligrams of quercetin per milliliter of extract (mg EQ/mL E). They were later converted to mg/g. The analyses were performed in triplicate.

Determination of total nitrogen and total protein

For the determination of total nitrogen and total protein, NMX-F-608-NORMEX-2011 (DOF, 2025) was taken into account using the Kjeldahl method. Approximately

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0.5 g of sample was weighed 2 Kjeltabs Cu/3.5 tablets were placed and 12 mL of concentrated sulfuric acid was added for digestion; it is then distilled with the addition of 80 mL of water, 30 mL of 40% sodium hydroxide and 50 mL of 4% boric acid receptor solution. Titration was performed with 0.01 N hydrochloric acid and Shiro Tashiro indicator until a color change. To obtain the results, the following equations were taken into account:

$$\% \text{ Nitrogen} = \underline{(Va - Vb) \times N \times 0.014 \times 100} / P \qquad \text{(Eq.1)}$$

$$\% \text{ Protein: } (\% \text{ Nitrogen})\ (F) \qquad \text{(Eq.2)}$$

where: Va = the volume spent on the degree; N = the normality of HCl hydrochloric acid; 0.014 = mEq of nitrogen; P = the weight of the sample; F = Conversion Factor.

Determination of ether extract

The determination was made using NMX-F-615-NORMEX-2018 (DOF, 2025). To determine the total fat content present, 2 grams of sample were taken and subjected to extraction with 120 mL of hexane, performing the total extraction of the fat-free matter in a reflux of 4 h. To obtain the results, *Equation 3* was taken into account:

$$\% \text{ lipids} = (c - d) - a / b \times 100 \qquad \text{(Eq.3)}$$

where: a = the constant weight of the flask; c = the weight of the flask with extract; b = the weight of the sample; d = the mass of the flask or solvent.

Determination of total biomass

At the end of the experiment, three plants were extracted from each tank to measure the total production of biomass. These plants' stems, leaves, and roots were dehydrated in a convection oven for three days at 60°C (Agraz Hernández et al., 2011).

Statistical analysis

To evaluate the effect of the herbicide 2,4-D dichlorophenoxyacetic acid on *Rhizophora mangle* seedlings, one-way analysis of variance (ANOVA) was applied to biochemical variables and biomass production. Subsequently, a post hoc test was applied using Fisher's minimum significant differentiation (LSD) method, with a significance level of $\alpha = 0.05$. Before this comparison analysis, the normality of the variables was validated with the method of Shapiro and Wilk (1965) with a significance level of $\alpha = 0.05$. As they did not meet the normal distribution assumption, the data were transformed using the Box-Cox method so that the variables present the assumptions of a normal distribution. Finally, a Pearson correlation analysis, principal component analysis, and simple linear regression were applied to determine the relationship between biochemical variables and biomass production. All statistical analyses were performed with a significance level of $\alpha = 0.05$, using Minitab 19 software (Minitab, Release 19.2020.1, LLC, in the United States). Sigma Plot 11.0 (SigmaPlot®, Release 11, San Jose, CA, USA) was used to create the graphs.

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Results

Physicochemical parameters before and during the application of the herbicide

During the seedling conditioning and afforestation phase (120 days), there was no significant variation in the physicochemical parameters of the interstitial water ($p > 0.05$). (*Fig. 3*). The interstitial water maintained a slightly alkaline (7.4 ± 0.2) and oxic (142 ± 16 mV) condition; temperatures ranged from (23 ± 2°C). The survival of seedlings during this phase was 100%. The physicochemical conditions of the interstitial water in the control units and those treated with the agrochemical 2,4-D showed a neutral pH with a slight tendency to alkalinity (7.2 ± 0.1 to 7.6 ± 0.3) and temperatures in a range of 29.0 ± 0.5 to 30 ± 1°C. The redox potential of the interstitial water was heterogeneous and oxic (156 ± 25 to -147 ± 142 mV) with significant differences between treatments (*Table 1,* $p < 0.05$).

Table 1. *One-way ANOVA of the physicochemical parameters of interstitial water in ponds during treatment with 2,4-D acid in Rhizophora mangle seedlings (p < 0.05)*

Parameters	gl (Factor, error)	Statistic F	P value
Temperature (°C)	(5, 18)	0.22	0.94
Redox Potential (mV)	(5, 18)	4.99	0.005
Salinity (UPS)	(5, 18)	0.77	0.58
pH	(5, 18)	1.78	0.17

Responses in biochemical variables

In general, exposure to different concentrations of 2,4-D caused an average decrease in biochemical variables in *Rhizophora mangle* seedlings where they were significantly lower in the concentration of total nitrogen (0.41 ± 0.01%), protein (2.6 ± 0.06%), flavonoids (0.75 ± 0.56 mg/g), compared to the control group with an average of 0.48 ± 0.01%, 2.9 ± 0.035% and 2.51 ± 1.5 mg/g respectively (*Fig. 4A, C, D*). However, the fat concentration presented a higher average concentration of 31.7 ± 5.8% among the exposed treatments compared to the control group of 8.58 ± 3.8% (*Fig. 4B*). The effect on the decrease in concentration in biochemical variables (total nitrogen, total protein, flavonoids) was noticeable from the exposure of 5 to 100% of the acid 2,4 D concerning the control (*Fig. 4A, C, D*), however, the increase in the concentration of fat was from the exposure of 5% to 100% of the 2,4-D, concerning control (*Fig. 4B*).

When performing a one-way ANOVA, significant differences were observed between the exposure treatments with 2,4-D acid on the flavonoid and

However, no significant differences were observed in the concentration of total nitrogen and total protein (*Table 2*).

Behavior of chlorophyll a concentration

In general, the average concentration of photosynthetic pigment (chlorophyll *a*) of plants treated with 2,4-D acid over the 12-day exposure time was significantly lower (60 ± 18.5 $\mu g\ g^{-1}$) compared to the control group (120 ± 10.1 $\mu g\ g^{-1}$) (*Fig. 4E*). The effect on chlorophyll *a* concentration was observed from exposure of 5% to 100% of 2,4 D acid (*Fig. 4E*). When performing a one-way ANOVA, significant differences were observed between 2,4-D acid exposure treatments in the concentration of chlorophyll *a* of *Rhizophora mangle* seedlings (*Table 2*).

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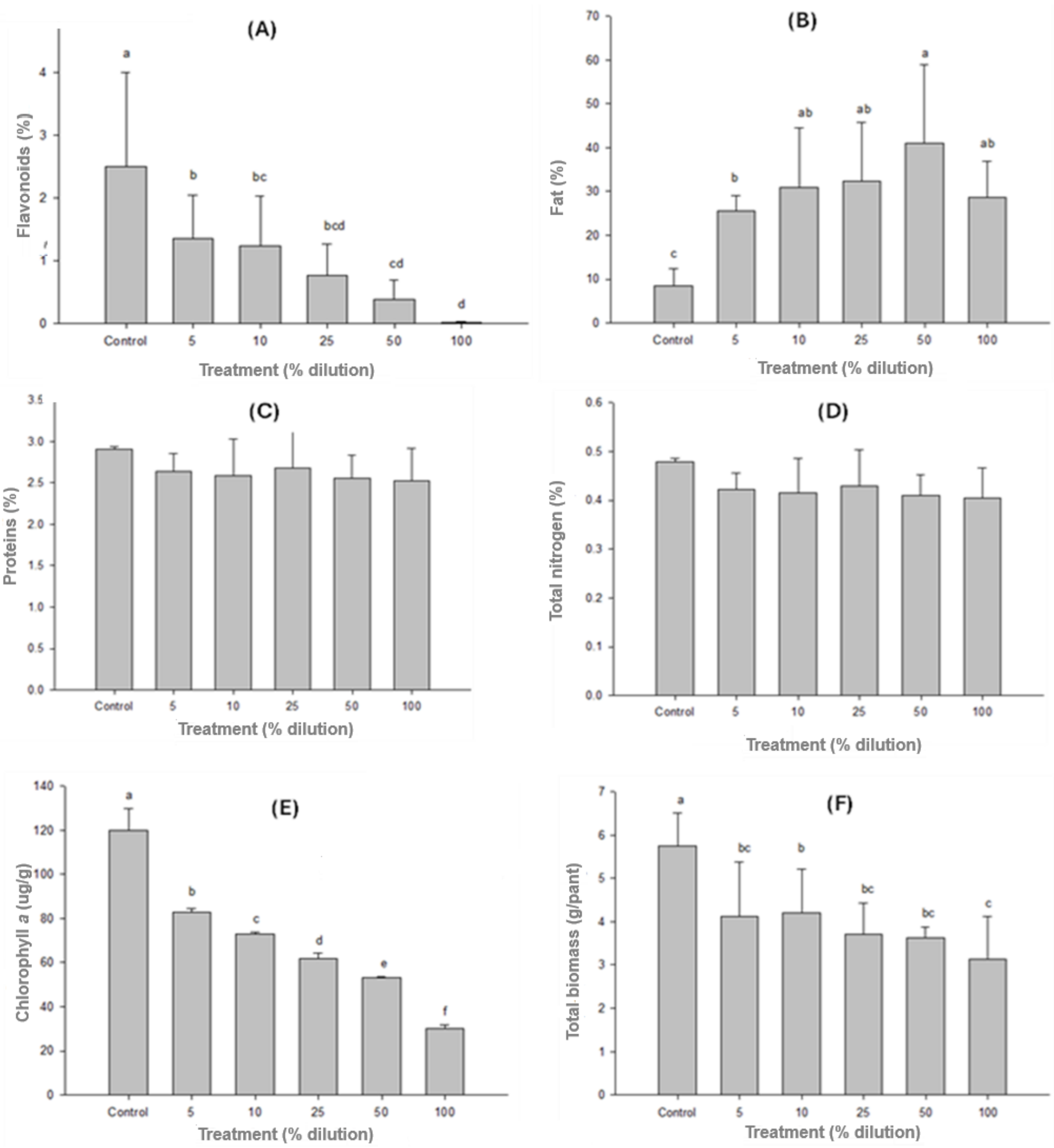

Figure 4. *Biochemical variables and total biomass production (mean ± standard deviation): (A) flavonoids; (B) fat; (C) total protein; (D) total nitrogen; (E) chlorophyll a; (F) total biomass of Rhizophora mangle seedlings among the treatments exposed. Treatments that do not share a letter are significantly different (p ≤ 0.05)*

Table 2. *Analysis of one-way variance concerning biochemical variables and total biomass of Rhizophora mangle seedlings exposed to different concentrations of 2,4-D acid (control, 5%, 10%, 25%, 50%, and 100%) with a significance level of p < 0.05*

Parameters	gl (Factor, error)	Statistic F	P value
Fat (%)	(5, 18)	5.36	0.003
Protein (%)	(5, 18)	0.99	0.453
Nitrogen (%)	(5, 18)	1.49	0.243
Flavonoids (mg/g)	(5, 18)	7.40	0.001
Chlorophyll a (µg/g)	(5, 18)	190.33	0.0001
Total biomass/plant (g)	(5, 18)	6.17	0.0001

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Simple linear regression analysis established an inverse relationship between 2,4-D concentrations and chlorophyll *a* concentration ($Y = 58.72 - 0.3883\ X$, $R^2 = 0.96$, $p \leq 0.003$).

Biomass production

Seedlings exposed to 2,4-D acid treatments showed a decrease in biomass production with a 35% decrease in total biomass production compared to the control (*Fig. 4F*). The effect on biomass production of *Rhizophora mangle* seedlings was observed from exposure of 5% to 100% of 2,4 D acid compared to the control (*Fig. 4F*). Simple linear regression analysis established an inverse relationship between 2,4-D concentrations and total biomass production of *R. mangle* seedlings ($Y = 4.17- 0.0107\ X$, $R^2 = 0.93$, $p \leq 0.008$). A one-way ANOVA analysis detected significant differences in biomass production of seedlings with treatment and control (*Table 2; Fig. 4F*).

Discussion

Description of the relationship of the behavior of biochemical parameters in R. mangle seedlings exposed to 2,4-D acid

The results of the principal component analysis (PCA) considering the biochemical parameters and the total biomass, exhibited 2 components that explain 96.6% of the total variance of the biochemical parameters in the *R. mangle* seedlings exposed to 2,4-D acid. Thus, in the first component (91.9%), he describes total biomass as the most important variable, showing a direct relationship between the concentration of total nitrogen, total protein, flavonoids, and chlorophyll *a* and an inverse relationship between fat concentration. The second component (4.7%) defines fat concentration as the important variable (*Fig. 5*). Likewise, this behavior was validated through a Pearson correlation between the biochemical variables and the total biomass of the *R. mangle* seedlings exposed to 2,4-D acid at different concentrations (*Table 3*).

Table 3. *Pearson correlation of biochemical variables and total biomass of R. mangle seedlings exposed to 2,4-D acid at different concentrations*

Correlation variables	r	P value
Protein (%) - Fat (%)	-0.844	0.035
Nitrogen (%)- Fat (%)	-0.862	0.027
Flavonoids (mg/g) - Fat (%)	-0.831	0.040
Chlorophyll *a* (µg/g) - Fat (%)	-0.783	0.066
Total biomass (g) - Fat (%)	-0.832	0.040
Nitrogen (%) - Protein (%)	0.997	0.000
Flavonoids (mg/g) - Protein (%)	0.896	0.016
Chlorophyll *a* (µg/g) - Protein (%)	0.898	0.015
Total biomass (g) - Protein (%)	0.921	0.009
Flavonoids (mg/g) - Nitrogen (%)	0.894	0.016
Chlorophyll a (µg/g) - Nitrogen (%)	0.892	0.017
Total biomass (g) - Nitrogen (%)	0.931	0.007
Chlorophyll a (µg/g) - Flavonoids (mg/g)	0.992	0.000
Total biomass (g) - Flavonoids (mg/g)	0.979	0.001
Total biomass (g) - Chlorophyll a (µg/g)	0.973	0.001

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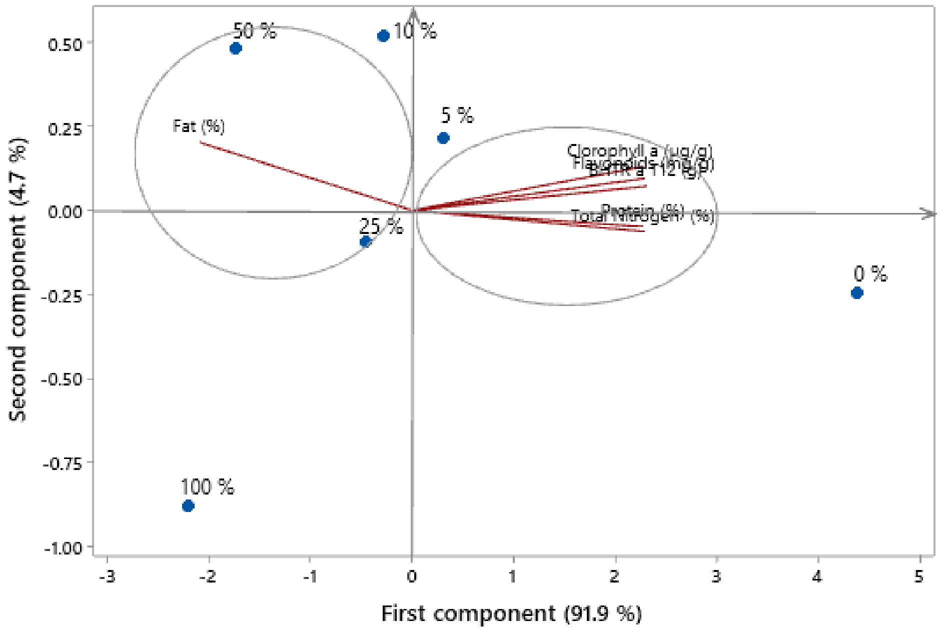

Figure 5. *Biplot of the principal component analysis (PCA) of axes 1 and 2, showing the blue dots, the exposure concentrations of the R. mangle seedlings and the red vectors representing the biochemical parameters*

The controlled conditions performed for this bioassay, as well as the emulation of the local tidal regime, favored the obtaining of better results. The physicochemical parameters of the interstitial water (pH, salinity, and temperature) during the experimental development did not show significant differences ($P > 0.05$) between the different treatments concerning the control, but not for the redox potential where significant differences were observed ($p < 0.05$), however, the values of variation of the redox potential of the experiment showed an oxic-type trend. Likewise, the values of variation of the redox potential between treatments may be due to the discharges of the 2,4-D solution at different concentrations during the process of recirculation and tidal emulation, as well as the physicochemical reaction of the mangrove sediment in the ponds. In this regard, Mitsch et al. (2000) and Gleason et al. (2003) mention that oxidation-reduction conditions are related to hydrology (water residence time, frequency of flooding due to tidal effects, and urban and/or industrial wastewater inputs) as well as the contributions of organic matter in mangrove forests where changes in redox potential are established. On the other hand, Agraz Hernández et al. (2018) experimented with the removal of nutrients from urban wastewater under controlled conditions similar to this study, these authors mention that the exposed conditions of controlled simulation favored the results of the bioassay due to the tidal emulation of the local regime and the ideal survival conditions of the *Rhizophora mangle* species.

In general terms, the biochemical variables (total nitrogen, total protein, flavonoids) of the *Rhizophora mangle* seedlings showed a decrease concerning the control, due to the effect of bioavailable 2,4-D in the interstitial water at different concentrations than that absorbed by the roots. In this regard, Parida et al. (2010) mention that 2,4-D can interfere with nitrogen metabolism in mangrove seedlings, altering the absorption and assimilation of nitrates, which affects the synthesis of amino acids and other essential nitrogenous molecules. Soligo et al. (2023) conducted a study on the effect of 2,4-D on nitrogen

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metabolism in wheat seedlings, showing as results that 2,4-D reduced nitrogen concentration in plants, which affected protein and amino acid synthesis, also found that this herbicide altered the activity of enzymes related to nitrogen metabolism. Similarly, Freiberg and Clark (1952) examined the effect of 2,4-D on nitrogen distribution in soybean plants, their results indicated that 2,4-D altered the distribution of nitrogen in plants, reducing the concentration of nitrogen in leaves and increasing it in roots. Likewise, Jayaraj et al. (2017) describes that 2,4-D can induce changes in protein synthesis and degradation in mangrove seedlings, affecting both the total amount of proteins and the expression of specific proteins involved in the stress response. On the other hand, Janeczko and Skoczowsk (2005) mention that seedlings exposed to 2,4-D can affect the synthesis of phenolic compounds and flavonoids, which are important in defense against stress and in cell signaling. On the other hand, the effect of 2,4-D on flavonoid composition and antioxidant activity in wheat seedlings has been determined. The results showed that 2,4-D altered the composition of flavonoids by increasing the concentration of some flavonoids and decreasing others, as well as affecting antioxidant activity (Liu et al., 2015).

Regarding the fat (lipid) variable, in general, higher production was observed in the treatments exposed to 2,4-D compared to the control where it was lower, this may be due to a stress mechanism in response to the higher production of lipids as a way of protecting the seedlings exposed to the treatments. As mentioned by Parida et al. (2010) who observed changes at the level of membrane lipid composition in *S. cerevisiae* cells adapted to 2,4-D, including an increase in the degree of membrane fatty acid saturation in a dose-dependent manner. On the other hand, Viegas et al. (2025) mentions that exposure to 2,4-D can alter the lipid composition in mangrove seedlings, affecting the integrity of cell membranes and the function of organelles. Lipids are crucial for the structure and function of plant cells, and any alteration can lead to cellular dysfunctions. Likewise, in conditions of herbicide-contaminated environments, mangrove seedlings can alter their metabolic pathway in the form of adaptability to the environment, as Moreira et al. (2020) say, where they observed that the free fatty acids of algae increased, especially when exposed to higher concentrations of fipronil and 2,4-D.

The effects on the decrease in chlorophyll *a* production in the leaves of seedlings treated in the two exposure times with 2,4-D are related to changes in physiological processes such as photosynthesis and stress due to the absorption of the herbicide affecting the pigmentation system of the seedlings. inhibition of photosynthetic efficiency and stages of seedling development (Filella et al., 1994; Haboudane et al., 2002; Wu et al., 2008). Similar findings have been reported by other authors such as Hallam (1970) and White and Hemphill (1972) where they describe the phytotoxic effect of 2,4-D on the leaves of tobacco seedlings and the decrease in the concentration of chlorophyll *a* is due to cell degeneration and degradation of the chlorophyll molecule. Filella et al. (1994) and Flores-de-Santiago et al. (2013), describe in a general way that mangroves are prone to natural or anthropogenic disturbances (stressed mangroves) where they experience decreases in the concentration of leaf chlorophyll and the production of leaf litter. On the other hand, an inverse relationship ($R^2 = 0.96$, $p \leq 0.003$) was determined between the concentrations of 2,4-D and the concentration of chlorophyll *a* in the leaves of *Rhizophora mangle* seedlings, similar results have been reported by Duke et al. (2005) where a high negative correlation ($R^2 = 0.99$, $p \leq 0.005$) was also determined between the concentration of the herbicide (diuron) in the sediment and concentration of chlorophyll *A* in the leaves of *Avicenia marina* trees, demonstrating the negative effect of herbicides on mangrove health in the Mackay region located in northeastern Australia which showed

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chlorosis and necrosis along the midveins of the leaves and only *A. corniculatum*, presented chlorosis and wilting of the leaves. The exposure time is a determining factor in the decrease in the amount of chlorophyll, when the seedling is exposed to the herbicide 2,4-D it causes prolonged stress (Badillo-Morales, 2023). Moreira, 2020 observed physiological (chlorophyll *a*) and morphological (cell size) changes in *R. subcapitata* cells when exposed to 2,4-D concentrations. The herbicide in the treatments at tested 2,4-D concentrations caused an increase in the cell size of the algae, compared to the control. Similar findings by Wong (2000) showed that the presence of 200 mg/L of 2,4-D completely inhibited algae growth, photosynthesis, and chlorophyll *a* synthesis.

Concerning total biomass production, we believe that the decrease in biomass production observed in *R. mangle* seedlings in treatments exposed to 2,4-D is due to the absorption and assimilation of the bioavailable 2,4-D herbicide in the interstitial water of the treated ponds. In this regard, Mendes et al. (2022) mentions that absorption through roots is the main route for herbicide transfer, where the influx of salts in mangroves is also regulated, i.e., the morphology, anatomy, and biomass production of roots is also associated with the absorption effect of herbicides, as presented in this study. The commercial 2,4-D herbicide is applied in high concentrations, and 2,4-D is absorbed through the roots, stems, and leaves where it is translocated to the meristems of the plant (Palma-Bautista et al., 2020; Song, 2013). The roots thicken and atrophy, the phloem and xylem on the stem disintegrate or become blocked and leaf growth ceases. Uncontrolled and unsustainable growth occurs, leading to stem curling, leaf wilting, decreased root size, and eventually plant death (Song, 2014). Bell et al. (2005) also mention that the cumulative effect of herbicide exposure on the roots and leaves of *A. corniculatum* seedlings is due to physiological stress, making it more sensitive. On the other hand, an inverse relationship was determined between the concentration of 2,4-D and the production of total biomass in *Rhizophora mangle seedlings* ($R^2 = 0.93$, $p \leq 0.008$), this may be due to the decrease in photosynthetic efficiency and the lower production of chlorophyll *a*, therefore affecting the production of the total biomass observed in this study. Moorthy and Kathiresan (1998) mention that the photosynthetic rate in mangroves depends on the concentration of photosynthetic pigments such as chlorophyll, and Kathiresan et al. (2018) determined a positive correlation between the carbon sequestration rate and the concentration of chlorophyll *a*, *b* and total in mangrove trees. Ashton and Crafts (1973) mention that one of the effects of herbicides on mangroves is direct interference with cell development division and differentiation. Likewise, Culic (1984) determined that contamination with 2,4-D in the communities of *Rhizophora stylosa* significantly inhibited the development of seedlings due to cell degeneration and described that the persistence of herbicide residues in the soil could inhibit the establishment and colonization of new mangrove seedlings.

The results of this bioassay were associated with simulated hydrology conditions, however, in the natural condition (environment), the factors that can influence the dispersion of 2,4-D are: the dilution rate of 2,4-D, the tidal frequency and the type of soil in mangrove forests. Moreover, this research demonstrated the adverse effects of 2,4-D exposure on *R. mangle* seedlings. For this reason, the regulation of this herbicide is considered fundamental and a priority for its rational use and even the prohibition of its use, recommending substitution of bioherbicides. Because the latter is more friendly to the environment, and the health of the population. Likewise, the acceptance and application of bioherbicides on a small, medium, and large scale would define an effective strategy for the conservation and recovery of the health of ecosystems and the ecosystem services they provide us.

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Conclusion

This study describes the response of the biochemical variables of *R. mangle* seedlings exposed to 2,4-D where it is related to adverse effects on the concentration of total nitrogen, total protein, and flavonoids, but it was not so with the concentration of fat where a higher concentration was observed compared to the control. Likewise, any of the concentrations of 2,4-D used in this experiment generated inhibitory effects on the production of chlorophyll and the total biomass of the seedlings that were exposed. Principal component analysis and Pearson's correlation showed an inverse relationship between biochemical variables (total nitrogen, total protein, flavonoids, and chlorophyll), total biomass production, and fat concentration. The oxic and physicochemical conditions presented during the development of the bioassay are generally those established in normal tidal regimes, which favored the obtaining of better results for this study in contrast to the reported experiments in which tides were not emulated. Based on the results obtained in this study, we could describe that pollution by wastewater discharges containing herbicides and poor unsustainable agricultural practices are worrying situations, since 2,4-D could affect the degree of health and permanence of mangroves, since at concentrations of 5% under experimental conditions they can alter the morphophysiological functions of the mangrove and therefore affect the environmental services it provides. We consider that other studies can be carried out on the phytotoxic effect of other commonly used herbicides using the same species, even other species and the number of individuals per afforestation impact areas, as well as the concentration of the herbicide to be applied.

REFERENCES

[1] Agraz Hernández, C. M., García Zaragoza, C., Iriarte-Vivar, S., Flores-Verdugo, F. J., Moreno Casasola, P. (2011): Forest structure, productivity and species phenology of mangroves in the La Mancha Lagoon in the Atlantic Coast of Mexico. – Wetl Ecol Manag 19: 273-293. DOI: 10.1007/s11273-011-9216-4.

[2] Agraz-Hernández, C. M., del Río-Rodríguez, R. E., Chan-Keb, C. A., Osti-Saenz, J., Muñiz-Salazar, R. (2018): Nutrient removal efficiency of *Rhizophora mangle (L.)* seedlings exposed to experimental dumping of municipal waters. – Diversity 10. DOI: 10.3390/D10010016.

[3] Alongi, D. M. (2002): Present state and future of the world's mangrove forests. – Environ Conserv 29: 331-349. DOI: 10.1017/S0376892902000231.

[4] Arellano-Aguilar, O., Rendón Von Osten, J. (2016): La Huella de Los Plaguicidas en México. – Grean Peace México A. C., México.

[5] Armitage, D., Alexander, S., Andrachuk, M., Berdej, S., Brown, S., Nayak, P., Pittman, J., Rathwell, K. (2017): Communities, multi-level networks and governance transformations in the coastal commons. – Governing the Coastal Commons 231-251. DOI: 10.4324/9781315688480-13.

[6] Ashton, F. M., Crafts, A. S. (1973): Mode of Action of Herbicides. – Wiley-Interscience, New York.

[7] Badillo-Morales, J. (2023): Impacto Del Tiempo de Exposición al Herbicida 2,4-D En La Cantidad de Clorofila y El Estrés Prolongado En Plántulas. – Revista de Fisiología Vegetal 58: 145-152.

[8] Bautista, F., Palacio, A. G. (2005): Caracterización y Manejo de Los Suelos de La Península de Yucatán: Implicaciones Agropecuarias, Forestales y Ambientales. First Ed. – Instituto Nacional de Ecología, Mexico.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_98179834

[9] Bell, A. M., Duke, N. C. (2005): Effects of photosystem ii inhibiting herbicides on mangroves—preliminary toxicology trials. – Mar Pollut Bull 51: 297-307. DOI: 10.1016/J.MARPOLBUL.2004.10.051.

[10] Beyond Pesticides (2025): Gateway on pesticide hazards and safe pest management. – https://www.beyondpesticides.org/resources/pesticide-gateway?pesticideid=1 (accessed on 17 March 2025).

[11] Chan-Keb, C. A., Agraz-Hernández, C. M., Muñiz-Salazar, R., Posada-Vanegas, G., Osti-Sáenz, J., Castellano, J. E. R., Conde-Medina, K. P., Vega-Serratos, B. E. (2018): Ecophysiological Response of *Rhizophora mangle* to the variation in hydrochemistry during five years along the Coast of Campeche, México. – Diversity 10. DOI: 10.3390/D10010009.

[12] Chan-Keb, C. A., Agraz-Hernández, C. M., Pérez-Balan, R. A., Gutiérrez-Alcántara, E. J., Muñiz-Salazar, R., Reyes-Castellano, J. E., Osti-Sáenz, J. (2021): Phytotoxicity in seedlings of *Rhizophora mangle (L.*) exposed to 2,4-dichlorophenoxyacetic acid under experimental conditions. – Journal of Marine Science and Engineering.

[13] Cissell, J. R., Delgado, A. M., Sweetman, B. M., Steinberg, M. K. (2018): Monitoring mangrove forest dynamics in Campeche, Mexico, using Landsat satellite data. – Remote Sens Appl 9: 60-68. DOI: 10.1016/J.RSASE.2017.12.001.

[14] CONANP (2006): PROGRAMA de conservación y manejo. – Reserva de La Biosfera Los Petenes, México.

[15] Culic, P. (1984): The Effects of 2,4-D on the Growth of *Rhizophora stylosa* Griff. Seedlings. – In: Teas, H. J. (eds.) Physiology and Management of Mangroves. Tasks for Vegetation Science. Springer, Dordrecht, pp. 57-63.

[16] DOF (2025a): Diario Oficial de la Federación NMX-F-608-NORMEX-2011. – https://dof.gob.mx/nota_detalle.php?codigo=5208839&fecha=12/09/2011#gsc.tab=0 (accessed on 17 March 2025).

[17] DOF (2025b): Diario Oficial de la Federación NMX-F-615-NORMEX-2018. – https://www.dof.gob.mx/nota_detalle.php?codigo=5565079&fecha=05/07/2019#gsc.tab=0 (accessed on 17 March 2025).

[18] Duke, N. C., Bell, A. M., Pederson, D. K., Roelfsema, C. M., Nash, S. B. (2005): Herbicides implicated as the cause of severe mangrove dieback in the Mackay Region, NE Australia: consequences for marine plant habitats of the GBR World Heritage area. – Mar Pollut Bull 51: 308-324. DOI: 10.1016/J.MARPOLBUL.2004.10.040.

[19] Filella, I., Penuelas, J. (1994): The red edge position and shape as indicators of plant chlorophyll content, biomass and hydric status. – Int J Remote Sens 15: 1459-1470. DOI: 10.1080/01431169408954177.

[20] Flores-de-Santiago, F., Kovacs, J. M., Flores-Verdugo, F. (2013): The influence of seasonality in estimating mangrove leaf chlorophyll-a content from hyperspectral data. – Wetl Ecol Manag 21: 193-207. DOI: 10.1007/S11273-013-9290-X/FIGURES/3.

[21] Freiberg, S. R., Clark, H. E. (1952): Effects of 2,4-dichlorophenoxyacetic acid upon the nitrogen metabolism and water relations of soybean plants grown at different nitrogen levels. – Botanical Gazette 113: 322-333. DOI: 10.1086/335724.

[22] Freisthler, M. S., Robbins, C. R., Benbrook, C. M., Young, H. A., Haas, D. M., Winchester, P. D., Perry, M. J. (2022): Association between increasing agricultural use of 2,4-d and population biomarkers of exposure: findings from the national health and nutrition examination survey, 2001-2014. – Environmental Health 21: 23. DOI: 10.1186/s12940-021-00815-x.

[23] Gill, A. M., Tomlinson, P. B. (1977): Studies on the growth of red mangrove (Rhizophora mangle L.) 4. The adult root system. – Biotropica 9: 145. DOI: 10.2307/2387877.

[24] Giri, C., Ochieng, E., Tieszen, L. L., Zhu, Z., Singh, A., Loveland, T., Masek, J., Duke, N. (2011): Status and distribution of mangrove forests of the world using earth observation satellite data. – Global Ecology and Biogeography 20: 154-159. DOI: 10.1111/J.1466-8238.2010.00584.X.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_98179834

[25] Gleason, S. M., Ewel, K. C., Hue, N. (2003): Soil redox conditions and plant–soil relationships in a Micronesian mangrove forest. – Estuar Coast Shelf Sci 56: 1065-1074. DOI: 10.1016/S0272-7714(02)00307-4.

[26] Haboudane, D., Miller, J. R., Tremblay, N., Zarco-Tejada, P. J., Dextraze, L. (2002): Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. – Remote Sens Environ 81: 416-426. DOI: 10.1016/S0034-4257(02)00018-4.

[27] Hallam, N. D. (1970): The effect of 2,4-dichlorophenoxyacetic acid and related compounds on the fine structure of the primary leaves of Phaseolus vulgaris. – J Exp Bot 21: 1031-1038.

[28] Hansmann, E., America, P. S. (1973): Handbook of Phycological Methods: Culture Methods and Growth Measurements. – In: Stein, J. R. (ed.) Handbook of Phycological Methods: Culture Methods and Growth Measurements. Dr. Malvern Gilmartin Collection. Cambridge University Press, Cambridge.

[29] Ivens, G. W. (1957): Arboricides for killing mangroves. – West African Rice Research Station Periodical Science Report 8.

[30] Janeczko, A., Skoczowski, A. (2005): Influence of 2,4-D on phenolic and flavonoid compound synthesis in seedlings. – Plant Growth Regul 46: 59-70.

[31] Jayaraj, J., Jayaraman, J., Janarthanan, S., Jeevanandam, M. (2017): Changes in protein synthesis and degradation induced by 2,4-d in magroves seedlings. – J Plant Physiol 221: 85-92.

[32] Kathiresan, K., Saravanakumar, K., Asmathunisha, N., Anburaj, R. (2018): Biochemical markers for carbon sequestration in two mangrove species (*Avicennia marina* and *Rhizophora mucronata*). – Beni Suef Univ J Basic Appl Sci 7: 733-739. DOI: 10.1016/j.bjbas.2018.10.003.

[33] Lee, S. Y. (1999): Tropical mangrove ecology: physical and biotic factors influencing ecosystem structure and function. – Australian Journal of Ecology 24: 355-366. DOI: 10.1046/J.1442-9993.1999.00984.X.

[34] Liu, Y., Zhang, J., Wang, X. (2015): Changes in flavonoid composition and antioxidant activity in wheat seedlings treated with 2,4-D. – J Agric Food Chem 63: 533-538.

[35] MacFarlane, G. R. (2002): Leaf biochemical parameters in Avicennia marina (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems. – Mar Pollut Bull 44: 244-256. DOI: 10.1016/S0025-326X(01)00255-7.

[36] Medina Méndez, A. J., Alejo Santiago, G., Soto Rocha, J. M., Hernández Pérez, M. (2018): Rendimiento de Maíz Grano Con y Sin Fertilización En El Estado de Campeche. – Rev Mex De Cienc Agric 0: 4306-4316. DOI: 10.29312/remexca.v0i21.1532.

[37] Mendes, K., Cabral Mielke, K., Dantonino, L., Silva, A. (2022): Retention, Absorption, Translocation, and Metabolism of Herbicides in Plants. – In: Mendes, K.F., Alberto da Silva, A. (eds.) Applied Weed and Herbicide Science. Springer, Cham, pp. 200-234.

[38] Mitsch, W. J., Gosselink, J. G. (2000): Wetlands. 3rd Ed. – John Wiley & Sons, New York.

[39] Moorthy, P., Kathiresan, K. (1997): Influence of ultraviolet-B radiation on photosynthetic and biochemical characteristics of a mangrove *Rhizophora apiculata*. – Photosynthetica 34: 465-471. DOI: 10.1023/A:1006828505050.

[40] Moreira, R. A., Rocha, G. S., da Silva, L. C. M., Goulart, B. V., Montagner, C. C., Melão, M. da G. G., Espindola, E. L. G. (2020): Exposure to environmental concentrations of fipronil and 2,4-D mixtures causes physiological, morphological and biochemical changes in *Raphidocelis subcapitata*. – Ecotoxicol Environ Saf 206: 111180. DOI: 10.1016/J.ECOENV.2020.111180.

[41] Naidoo, G. (2016): The mangroves of South Africa: an ecophysiological review. – South African Journal of Botany 107: 101-113. DOI: 10.1016/J.SAJB.2016.04.014.

[42] Pacheco, A. O., Moran, J. M., Rivero Breff, D., María, E., Saborít, A. (2013): Caracterización Física, Físico-Química y Química de Extractos Totales de Hojas Frescas de *Petiveria Alliacea L*. Con Acción Antimicrobiana. (Physical, physical-chemical and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_98179834

chemical characterization of total extracts of fresh leaves from *Petiveria alliacea L.* with antimicrobial action). – Rev Mex Cienc Farm 44.

[43] Palma-Bautista, C., Rojano-Delgado, A. M., Dellaferrera, I., Rosario, J. M., Vigna, M. R., Torra, J., De Prado, R. 0. (2020): Resistance mechanisms to 2,4-D in six different dicotyledonous weeds around the world. – Agronomy 10(4: 566. https://doi.org/10.3390/agronomy10040566.

[44] Parida, A. K., Jha, B. (2010): Salt tolerance mechanisms in mangroves: a review. – Trees 24: 199-217. DOI: 10.1007/s00468-010-0417-x.

[45] Pazmiño, D. M., Rodríguez-Serrano, M., Romero-Puertas, M. C., Archilla-Ruiz, A., Del Río, L. A., Sandalio, L. M. (2011): Differential response of young and adult leaves to herbicide 2,4-dichlorophenoxyacetic acid in pea plants: role of reactive oxygen species. – Plant Cell Environ 34: 1874-1889. DOI: https://doi.org/10.1111/j.1365-3040.2011.02383.x.

[46] Peng, L., Wenjian Z., Zhenji, L. (1997): Distribution and accumulation of heavy metals in A Vicennia Marina Communi Ty in Shenzhen, China. – Journal of Environmental Science 9: 472-479.

[47] Peters, E. C., Gassman, N. J., Firman, J. C., Richmond, R. H., Power, E. A. (1997): Ecotoxicology of tropical marine ecosystems. – Environ Toxicol Chem 16: 12-40. DOI: 10.1002/ETC.5620160103.

[48] Rivera-Arriaga, E., Azuz-Adeath, I. A., Díaz Mondragón, S. (2021): Legal proposal modification analysis of the maritime terrestrial federal zone of Mexico. – Revista Costas 3(1): 91-110. DOI: 10.25267/ Costas0403

[49] Shapiro, S. S., Wilk, M. B. (1965): An analysis of variance test for normality (complete samples). – Biometrika 52: 591. DOI: 10.2307/2333709.

[50] Soligo, V., Galon, L., Forte, C. T., Brunetto, L., Silva, A. M. L. da Gallina, A., Perin, G. F. (2023): Does doses and time of 2,4-D application interfere in the physiology and wheat grains yield components? – Arq Inst Biol (Sao Paulo) 89: e00342020. DOI: 10.1590/1808-1657000342020.

[51] Song, Y. (2013): Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. – J Integr Plant Biol 56. DOI: 10.1111/jipb.12131.

[52] Song, Y. (2014): Uncontrolled and unsustainable growth induced by 2,4-D: stem curling, leaf wilting, root size reduction, and plant death. – Journal of Plant Biology 57: 213-220.

[53] Strickland, J. D. H., Parsons, T. R. (1965): A Manual of Seawater Analysis. Vol. 125. – Fisheries Research Board of Canada, Ottawa.

[54] Teas, H. J. (1976): Herbicide Toxicity in Mangroves. – EPA-600/3-76-004, Florida.

[55] Troche-Souza, C., Rodríguez-Zúñiga, M. T., Velázquez-Salazar, S., Valderrama-Landeros, L., Villeda-Chavéz, E., Alcántara-Maya, A., Vázquez-Balderas, B., Cruz-López, M. I., Ressl, R. (2017): Manglares de México: Extensión, Distribución y Monitoreo (1970/1980–2015). First Ed. – Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Mexico.

[56] Vázquez-Lule, A., Colditz, R., Herrera-Silveira, J., Guevara, M., Rodríguez-Zúñiga, M. T., Cruz, I., Ressl, R., Vargas, R. (2019): Greenness trends and carbon stocks of mangroves across Mexico. – Environmental Research Letters 14: 075010. DOI: 10.1088/1748-9326/ab246e.

[57] Velázquez-Salazar, S., Rodríguez-Zúñiga, M. T., Alcántara-Maya, J. A., Villeda-Chávez, E., Valderrama-Landeros, L., Troche-Souza, C., Vázquez-Balderas, B., Pérez-Espinosa, I., Cruz-López, M. I., Ressl, R. (2021): Manglares de México. Actualización y Análisis de Los Datos 2020. – Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México.

[58] Viegas, C. A., Cabral, M. G., Teixeira, M. C., Neumann, G., Heipieper, H. J., Sá-Correia, I. (2005): Yeast adaptation to 2,4-dichlorophenoxyacetic acid involves increased membrane fatty acid saturation degree and decreased OLE1 transcription. – Biochem Biophys Res Commun 330: 271-278. DOI: 10.1016/J.BBRC.2005.02.158.

[59] White, J. A., Hemphill, D. D. (1972): An ultrastructural study of the effects of 2,4-D on tobacco leaves. – Weed Sci 20: 478-481. DOI: 10.1017/S0043174500036183.
[60] WHO (2020): The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2009. – WHO, Geneva.
[61] Wong, P. K. (2000): Effects of 2,4-D, glyphosate and paraquat on growth, photosynthesis and chlorophyll–a synthesis of Scenedesmus quadricauda Berb 614. – Chemosphere 41: 177-182. DOI: 10.1016/S0045-6535(99)0040
[62] Wu, C., Niu, Z., Tang, Q., Huang, W. (2008): Estimating chlorophyll content from hyperspectral vegetation indices: modeling and validation. – Agric For Meteorol 148: 1230-1241. DOI: 10.1016/J.AGRFORMET.2008.03.005.
[63] Zheng, G. J., Lam, M. H. W., Lam, P. K. S., Richardson, B. J., Man, B. K. W., Li, A. M. Y. (2000): Concentrations of persistent organic pollutants in surface sediments of the mudflat and mangroves at Mai Po Marshes Nature Reserve, Hong Kong. – Mar Pollut Bull 40: 1210-1214. DOI: 10.1016/S0025-326X(00)00190-9.

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